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(71)Applicant : NATIONAL CARDIOVASCULAR CENTER
CHUGAI PHARMACEUT CO LTD
(72)Inventor : SAWAMURA TATSUYA
SUZUKI HIROSHI
KAWASE YOSUKE

(54) LOX-1 GENE DEFECTIVE ANIMAL

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a gene-modified animal in which the expression of a LOX-1 gene pair is inactivated, capable of being used for elucidating physiological activities of the LOX-1 in an individual body level and enabling the utilization of the same for a research on a therapeutic agent, etc., of diseases including arteriosclelosis, etc., associated with the gene.
SOLUTION: This LOX-1 defective mouse is produced by a gene-targeting method. By an analysis using the produced LOX-1 gene defective mouse, it becomes clear that a LOX-1 antagonist is useful for improving a morbid state caused by the lowering of endothelial function. Also, it becomes clear that the LOX-1 is associated with an infection with a protozoan. Thus, the LOX-1 gene defective animal is a useful model for elucidating a new function of the LOX-1. The knock out mouse of the function of the LOX-1 gene becomes a useful model mouse for exploring the unknown role of the LOX-1 gene in the onset and development of the arteriosclerosis, etc.

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CLAIMS

[Claim(s)]
[Claim 1]
The embryonic stem cell by which LOX-1 gene expression was controlled.
[Claim 2]
The embryonic stem cell according to claim 1 by which LOX-1 gene expression is controlled when the gene of one side of a LOX-1 gene pair or both suffers a loss.
[Claim 3]
The embryonic stem cell according to claim 1 which is what the deficit of a gene depends on insertion of a foreign gene.
[Claim 4]
The embryonic stem cell according to claim 1 whose embryonic stem cell is a cell of the rodent origin.
[Claim 5]
The nonhuman animal by which LOX-1 gene expression was controlled.
[Claim 6]
The nonhuman animal according to claim 5 by which LOX-1 gene expression is controlled when the gene of one side of a LOX-1 gene pair or both suffers a loss.
[Claim 7]
The nonhuman animal according to claim 5 which is what the deficit of a gene depends on insertion of a foreign gene.
[Claim 8]
The nonhuman animal according to claim 5 whose nonhuman animal is rodent.

[Claim 9]
The following processes are included. LOX-1 agonist and/or the screening approach of an accelerator,
(1) The process which infects the Babesia protozoa with any 1 term of

claims 5-8 to the nonhuman animal of a publication
The process which medicates the animal of (2) and (1) with an examined substance
(3) The process which chooses the examined substance which makes the symptom of the infectious disease by protozoa ease
[Claim 10]
The constituent for a protozoan disease therapy which makes an active principle LOX-1 agonist and/, an accelerator, or a manifestation accelerator.
[Claim 11]
The ischemic disease which makes an active principle LOX-1 antagonist and/, an inhibitor, or a LOX-1 manifestation inhibitor, hyperlipidemia, diabetes mellitus, and the constituent for a hypertension disease therapy.
[Claim 12]
The anti-inflammatory agent which makes an active principle LOX-1 antagonist and/, an inhibitor, or a LOX-1 manifestation inhibitor.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]
This invention relates to those use at the cell by which LOX-1 gene was knocked out and an animal, and a list.
[0002]
[Description of the Prior Art]
The malfunction of a vascular endothelial cell has close relation with various symptoms including arteriosclerosis. The fall of the blood vessel relaxation operation observed by arteriosclerosis, hypertension and a diabetic model animal, and the list through a patient's blood vessel is considered that reduction of the nitrogen-monoxide (NO) emission from a vascular endothelial cell is the cause. Moreover, it is known that secretion of the chemotactic factor from a vascular endothelial cell and a manifestation rise of an adhesion factor will participate in infiltration of the macrophage as which an arteriosclerosis blow hole tries to be sufficient. Although it is thought that various stimuli are participating in change of these endothelial cells It is already shown that change of the above endothelial cells is guided with the oxidized low density lipoprotein (LDL; low density lipoprotein). Attention It is collecting (). [Oka K. et al.] (1998) Proc. Natl. Acad. Sci. USA 95 : 9535-40; Kakutani M. et al. (2000) Proc. Natl. Acad. Sci. USA 97 : 360-4.
[0003]
Cloning of the oxidation LDL (following, oxLDL) acceptor (LOX-1; lectin oxidized low density lipoprotein receptor-1) is carried out as one of the oxLDL acceptors in a vascular endothelial cell. (Sawamura T. et al. (1997) Nature 386: 73-7) It is shown clearly that it exists in a macrophage and a vascular endothelial cell. In the vascular endothelial cell, a manifestation is guided to oxLDL and a list by arteriosclerosis related factors, such as hyperlipidemia, hypertension, and diabetes mellitus, and, as for LOX-1, it is shown that the manifestation is accelerating in an arteriosclerosis blow hole. It is a well-known fact that incorporation of oxLDL through LOX-1 guides endothelial cell death. Identification of structural analysis of Homo sapiens LOX-1 gene and the physiological ligand of LOX-1 has also already been performed. Moreover, it is shown that NO production incompetence in the endothelial cell by oxLDL originates in inhibition of high compatibility arginine transportation and production of active oxygen. Furthermore, the possibility that LOX-1 antagonist is effective in diseases, such as

arteriosclerosis, is also suggested from the experiment using the neutralizing antibody to LOX-1.

[0004]

The device which minded [of the foreign matter recognition and incorporation of a macrophage] immunomechanism, and the device which is not minded are known. The device which does not mind the latter immunomechanism is also called a scavenger device, and the I-beam and the II mold class A macrophage scavenger receptor (SR-AI/II) are known as the typical acceptor. It is the protein with which LOX-1 as well as SR-AI/II belongs to a scavenger receptor family. About SR-AI/II, the onset of arteriosclerosis and progress, the phylaxis of a pathogenic microbe, Removal of foreign matter wastes, Participating in a protozoan disease is reported (147 (2000); J.Exp. Med. 186:). [Nature 386:] [292 (1997);J. Exp. Med. 191:] 1431 (1997); J. Protozool. Res. 7 : 81 (1997); Am. J. Trop. Med. Hyg. 59 : 843 (1998) . Therefore, possibility of participating in these diseases and infectious diseases can be considered like [LOX-1] SR-AI/II.

[0005]

[Nonpatent literature 1]

Sawamura T. et al. (1997) Nature 386: 73-7

[0006]

[Problem(s) to be Solved by the Invention]

If the gene alteration animal by which the manifestation of a LOX-1 gene pair was inactivated can be obtained, the physiological function of LOX-1 can be solved on individual level, and it will also become possible to use for the research of the remedy of an ischemic disease including arteriosclerosis etc. relevant to this gene. Moreover, the mouse with which the function of such LOX-1 gene was knocked out turns into a useful model mouse, when exploring the role of the onset of arteriosclerosis, progress, and other LOX-1 strange genes.

[0007]

[Means for Solving the Problem]

Then, in order to explore the function of LOX-1 gene, production of a LOX-1 genetic-defect mouse (following and LOX-1KO mouse) was tried. Various examination was performed, as the example of this specification was shown, cloning of the mouse LOX-1 gene was carried out, the vector for homologous recombination was built, the embryonic stem cell which has variation LOX-1 gene by homologous recombination using this vector was established, the chimera germ was produced, and it succeeded in obtaining a gay deficit mouse by crossing a hetero deficit mouse. The following functions were able to be checked by using this mouse.

[0008]

(1) The function in infection

The Babesia rodhaini infection experiment to a LOX-1 genetic defect (KO) mouse was conducted. Consequently, although the difference was not accepted between KO mouse and the wild type mouse about the survival rate after Babesia rodhaini (10,000 IRBC (infected red blood cell; infection erythrocyte)) infection, the inclination to go up an early stage and more quickly than that of PARASHITEMIA, and a significant reduction of a hematocrit value were observed with KO mouse. Moreover, in the Babesia microti (1x107 IRBC) infection to KO mouse, changing to 3 **** was observed with KO mouse to the peak of bimodal PARASHITEMIA being shown with the wild type mouse. The difference with a wild type mouse was not accepted in transition of a

hematocrit value. As mentioned above, the results which suggest the intervention to this protozoan disease of Lox-1 were acquired by the infection experiment of the Babesia protozoa which are parasitic on an erythrocyte like malaria.

[0009]

(2) Blood vessel inner-bark function

Using Prostaglandin F (PGF) 2alpha and acetylcholine which are vasoexcitor material, in the mouse of this invention, blood vessel inner-bark functional evaluation was performed, and it became clear that an inner-bark functional trauma operation of oxLDL is due to the signal through LOX-1. And it was thought that LOX-1 antagonist and/or an inhibitor rose an inner-bark function, and it was shown that LOX-1 antagonist is useful to the improvement of the symptoms to which the inner-bark function fell.

[0010]

(3) The function in inflammation

The operation over the carrageenin guide-peg edema in a LOX-1 genetic defect (KO) mouse and a normal mouse was considered, and it was shown by the mouse with which LOX-1 gene was knocked out from the rate of an edema, and the pathology organization view that an inflammatory response is controlled compared with a normal mouse.

[0011]

Consequently, although the agonist and/or the accelerator of LOX-1 deal with a protozoan disease in that LOX-1 antagonist and/or an inhibitor are useful although it deals with an ischemic disease, hyperlipidemia, diabetes mellitus, and a hypertension disease, and a list, the useful thing became clear. Moreover, by using the transgenic animal which suffers a loss in LOX-1 gene, these useful antagonists and/or the inhibitor, and the list were realized [that agonist and/or an accelerator can be screened and], and this invention was completed. This invention is in a detail more,

[1] The embryonic stem cell by which LOX-1 gene expression was controlled,

[2] the above [1] by which LOX-1 gene expression is controlled when the gene of one side of a LOX-1 gene pair or both suffers a loss -- a publication -- an embryonic stem cell

[3] The embryonic stem cell of the above-mentioned [1] publication which is what the deficit of a gene depends on insertion of a foreign gene,

[4] The embryonic stem cell of the above-mentioned [1] publication whose embryonic stem cell is a cell of the rodent origin,

[5] The nonhuman animal by which LOX-1 gene expression was controlled,

[6] LOX - one -- a gene -- a pair -- one side -- or -- both -- a gene -- suffering a loss -- having -- things -- LOX - one -- gene expression -- controlling -- having -- **** -- the above -- [-- five --] -- a publication -- nonhuman -- an animal

[7] The nonhuman animal of the above-mentioned [5] publication which is what the deficit of a gene depends on insertion of a foreign gene,

[8] The nonhuman animal of the above-mentioned [5] publication whose nonhuman animal is rodent,

[9] Include the following processes. LOX-1 agonist and/or the screening approach of an accelerator,

(1) The above [5] Process which infects the Babesia protozoa with either of - [8] to the nonhuman animal of a publication

The process which medicates the animal of (2) and (1) with an examined substance

(3) The process which chooses the examined substance which makes the symptom of the infectious disease by protozoa ease
[10] The constituent for a protozoan disease therapy which makes LOX-1 agonist and/or an accelerator an active principle,
[11] In the ischemic disease which makes an active principle LOX-1 antagonist and/, an inhibitor, or a LOX-1 manifestation inhibitor, hyperlipidemia, diabetes mellitus and the constituent for a hypertension disease therapy, and a list
[12] LOX-1 antagonist and/, an inhibitor, or the anti-inflammatory agent that makes a LOX-1 manifestation inhibitor an active principle
It is alike and is related.
[0012]
[Embodiment of the Invention]
The gene alteration embryonic stem cell and gene alteration animal of this invention are characterized by controlling LOX-1 gene expression artificially. Cloning of the LOX-1 gene has already been carried out as one of the scavenger receptors. (Sawamura T. et al. (1997) Nature386: 73-7) . the base sequence of mouse LOX-1 gene, and the amino acid sequence of the protein by which a code is carried out to it -- respectively -- array number: -- it is shown as 1 and 2.
[0013]
Although especially the animal set as the object of an alteration of LOX-1 gene in this invention is not limited, they are rodents, such as a mouse, a rat, and a hamster, preferably. A mouse is desirable when taking into consideration using as animal model especially. Moreover, although especially the embryonic stem cell set as the object of an alteration of LOX-1 gene is not limited, either, rodent and the thing which originates in a mouse especially are desirable. An embryonic stem cell can be acquired by the well-known approach, and can be maintained (Doetschman et al. (1985) J. Embryol. Exp. Morphol. 87: refer to 27-45).
[0014]
Here, the condition that only one manifestation of a gene pair besides the perfect control (null allele) by which the manifestation of both LOX-1 gene pairs was controlled ["LOX-1 gene expression was controlled" and] was controlled and of having been controlled imperfectly is included. In this invention, it is desirable that LOX-1 gene expression is controlled specifically. In the gene alteration embryonic stem cell and animal of this invention, the approach of making the approach and LOX-1 gene-expression regulatory region which make LOX-1 gene suffering a loss suffering a loss as a means to control LOX-1 gene expression artificially can be mentioned. As the deficit approach of an internality gene and manifestation regulatory region, although a gene targeting can be considered, the approach of inserting a foreign gene in desirable one side or desirable both of a LOX-1 gene pair, and inactivating a gene can be mentioned.
[0015]
LOX-1 gene-expression regulatory region can be obtained from genomic DNA by the well-known approach using the base sequence of LOX-1. How to specify a transcription initiation site like S1 mapping (the volume cell technology separate volume 8 new cell technology protocols and for University of Tokyo Institute of Medical Science carcinostatic research sections (1993) 362-374 pages, Shujunsha) It is available. Generally, a genomic DNA library can be preferably screened by the ability making

30-50bp into probe DNA 15 to 100 bp, and gene expression regulatory region can shorten or fragment the obtained polynucleotide of a five prime end, and can acquire it by investigating the manifestation ability using a reporter gene. Furthermore Neural Networkhttp://www.fruitfly.org./seq_tools/promoter.html; Reese et al. (1996) Biocomputing: Proceedings of the 1996 Pacific Symposium Hunter and Klein ed. World Scientific Publishing Program and it reaches for predicting gene expression regulatory region, such as Co. The program (http://biosci.cbs.umn.edu./software/proscan/promoterscan.htm) which predicts the activity smallest unit of manifestation regulatory region is also well-known, and can be used.

[0016]

The genetically engineered mouse of this invention is the following, and can be made and created. First, DNA containing the exon part of Shinsei LOX-1 gene is isolated from a mouse, the suitable gene for this DNA fragment is inserted, and a targeting vector is built. As a gene inserted in a DNA fragment, use of the marker gene of antibiotic resistance genes (neomycin resistance gene etc.) which can check installation of this vector can be considered, for example. When an antibiotic resistance gene is used, it can be checked by cultivating a cell in the culture medium containing an antibiotic whether this vector has been introduced into the cell. By being inserted into a cell, a targeting vector is included in genomic DNA by homologous recombination so that the imprint of LOX-1 natural gene may be barred or it may prevent. next, this vector -- erection PORESHON -- it introduces into an embryonic stem cell stock by law, the microinjection method, a calcium phosphate method, etc., and the cell strain which produced homologous recombination is selected. In order to select a still more efficient recombination cell, the cell strain which caused non-homologous recombination can also be eliminated by combining a thymidine kinase gene etc. with a targeting vector. Moreover, a diphtheria toxin A fragmentation (DT-A) gene etc. is combined with a targeting vector, and how to eliminate the cell strain which caused non-homologous recombination is also learned, and in case the variant cell or animal of this invention is produced, it can use. Moreover, PCR and Southern blotting can perform homologous recombination assay, and the cell strain by which at least one side of a gene pair of LOX-1 gene was inactivated can also be obtained efficiently.

[0017]

When selecting the cell strain which produced homologous recombination, also as for fear of the gene disruption by gene insertion other than the part of homologous recombination, for a certain reason, it is desirable to produce a chimera using two or more clones. Injection of the obtained embryonic stem cell stock can be carried out to a mouse blastocyst, and a chimeric mouse can be obtained. By making this chimeric mouse cross, the mouse which inactivated one side of a gene pair of LOX-1 gene can be obtained. Furthermore, the mouse which inactivated both LOX-1 gene pairs can be obtained by making this mouse cross. Also in animals other than the mouse with which the embryonic stem cell is established, a LOX-1 gene alteration cell and an animal can be obtained by the same technique.

[0018]

Moreover, the embryonic stem cell stock with which both gene pairs of LOX-1 gene were inactivated is acquirable also by the following approaches. That is, the cell strain by which another side of a gene pair was also inactivated, i.e., the embryonic stem cell

stock with which both gene pairs of LOX-1 gene were inactivated, can be obtained by cultivating the embryonic stem cell stock which inactivated one side of a gene pair by the culture medium containing a high-concentration antibiotic. Moreover, also when the embryonic stem cell stock which inactivated one side of a gene pair is selected, a targeting vector is again introduced into this cell strain and the cell strain which produced homologous recombination chooses, the embryonic stem cell stock with which both gene pairs of LOX-1 gene were inactivated can be obtained. When performing homologous recombination for the second time, it is desirable to use what is different in the marker gene inserted in a targeting vector by the first object for homologous recombination and the object for homologous recombination of a two-times eye.

[0019]

A well-known approach can be used as an approach of establishing the cell strain of the gene alteration animal origin of this invention. For example, the approach of the primary culture of a fetus cell can be used in rodent (a new chemistry experiment lecture, 18 volumes, 125-129 pages, Tokyo Kagaku Dojin; reference, such as an operating manual of a mouse germ, 262-264 pages, and modern publication).

[0020]

Relation with the blood vessel inner-bark function of LOX-1, protozoal infection, and an inflammatory response was shown by the analysis using the LOX-1 genetic-defect mouse of this invention (six to example 8 reference). Therefore, a LOX-1 genetic-defect animal is not only applicable to the analysis of the detailed function of LOX-1 gene, but it can use it for the onset and progress of the disease in which the fall of blood vessel inner-bark functions, such as hyperlipidemia, diabetes mellitus, a hypertension disease, and an ischemic disease, participates, protozoal infection, and a list as a model animal for elucidations, such as an inflammatory response.

[0021]

Furthermore, it can use also for screening of a LOX-1 agonist, an accelerator, etc. which serve as a candidate of a remedy to an infectious disease. That is, the screening approach of of the LOX-1 agonist and/or the accelerator which used the nonhuman animal by which the LOX-1 gene expression of this invention was controlled by this invention is offered. In a detail, LOX-1 agonist and/or an accelerator can be screened more by choosing the examined substance which infects the Babesia protozoa to the nonhuman animal by which the LOX-1 gene expression of (1) this invention was controlled, and (2) this animals are medicated [examined substance] with an examined substance, and makes the symptom of the infectious disease by (3) protozoa ease. Thus, LOX-1 agonist and/or the accelerator which were screened can be used as a constituent for a protozoan disease therapy.

[0022]

Although especially the approach of infecting the Babesia protozoa to the nonhuman animal by which LOX-1 gene expression was controlled is not limited, as A of an example 7 is shown, the approach of taking in an infection erythrocyte to an animal is employable, for example. Moreover, the "examined substance" in this invention may be what kind of compound. For example, the matter of libraries, such as a gene library, a synthetic low-molecular-weight-compound library, a synthetic peptide library, an antibody library, and a RANDAMUFA dipeptide display library, can be mentioned. In addition, the quality of a natural product can also be used as an examined substance. As quality of a natural product, bacteria secrete, a microorganism, vegetation, the

extract from the cell of an animal and the culture supernatant of these cells, soil, etc. can be illustrated.

[0023]

Although not limited to this, selection of the examined substance which makes the symptom of the infectious disease by protozoa ease can be performed by authorizing the capacity of an examined substance by investigating the inclination or hematocrit value of PARASHITEMIA, as an example is shown. It was shown clearly that the inclination for PARASHITEMIA to go up quickly compared with the animal of a wild type, and a hematocrit value decreased for a LOX-1 genetic-defect animal by this invention. Then, it is thought that the effectiveness which controls the rise of PARASHITEMIA in the animal by which the LOX-1 gene expression of this invention was controlled, or the examined substance which prevents or eases reduction in a hematocrit value is useful to the treatment of a protozoan disease.

[0024]

The agonist and/or the accelerator which were chosen by the screening approach using the nonhuman animal by which the LOX-1 gene expression of this invention was controlled can be prepared combining support, a medium, etc. which are permitted pharmacologically suitably. For example, a stabilizer, anti-oxidants (ascorbic acid etc.), a surfactant, buffers (a citric acid, a phosphoric acid, other organic acids, etc.), a chelating agent, corrigent, a binder, perfume, a physiological saline, an isotonic solution, an excipient, antiseptics, a solubilizing agent, etc. can be blended. Moreover, amino acid, such as an asparagine, an arginine, a glycine, a glutamine, and a lysine, serum albumin, gelatin, a carbohydrate, protein, a low-molecular-weight polypeptide, sugar-alcohol (a mannitol, sorbitol, etc.), a saccharide (a polysaccharide and monosaccharide), an immunoglobulin, etc. may also be included as other matter if needed. When considering as the water solution for injection, it is desirable to mix with the isotonic solution (for example, thing containing D-sorbitol, D-mannose, D-mannitol, and a sodium chloride) containing the adjuvant of a physiological saline, grape sugar, or others, and it may use together with a still more suitable solubilizing agent (ethanol etc.), for example, alcohol, polyalcohols (propylene GUKORU, PEG, etc.), a nonionic surfactant (polysorbate 80, HCO-50 grade), etc.

[0025]

Moreover, it can also be used if needed, being able to enclose with a microcapsule. As a microcapsule, what consists of a hydroxymethyl cellulose, gelatin, Pori [methyl methacrylic-acid], etc., for example is well-known. Moreover, the colloid drug delivery system (refer to Remington's Pharmaceutical Scinece 16th ed. and Oslo ed. (1980)) using liposome, an albumin microsphere, microemulsion, a nano particle, a nano capsule, etc. is also available. In addition, the approach of preparing as sustained-release drugs is also well-known (for example). Langer et al. (1981) J. Biomed.Mater. Res. 15 : 167-277; Langer (1982) Chem. Tech. 12 : 98-105; Sidman et al. (1983) Biopolymers 22 : 547-56; US3,773,919; EP58,481; It is referring to the EP133,988 grade. It can apply to the constituent and drugs of this invention. Moreover, when the code of this agonist and the accelerator may be carried out by the gene, it is also possible to perform a nest and gene therapy for the gene which carries out the code of this agonist or the accelerator to the well-known vector for gene therapies.

[0026]

Administration to a patient is preferably performed by injection and intravenous drip,

and intraarterial injection, an intravenous injection, and subcutaneous injection are mentioned. In addition, it is also possible to pass and to prescribe a medicine for the patient according to paths, such as transderma and taking orally, in a bronchial tube and a muscle in a nasal cavity. Although a dose changes with classes, routes of administration, etc. of the class of a patient's weight and age, and illness with which it deals and a symptom, and the drugs to be used, if it is this contractor, it can choose the suitable dose according to each situation suitably.

[0027]

It became clear that that an inner-bark function rises by the deficit of LOX-1, an edema, and inflammation were controlled by the experiment which used the LOX-1 genetic-defect animal of this invention. That is, if the matter which controls the manifestation of LOX-1 in the living body can be obtained, it is useful as the remedy and anti-inflammatory agent of the symptoms in which the fall of an inner-bark function participates. Then, this invention offers the screening approach of a LOX-1 manifestation inhibitor. LOX-1 antagonist and/or an inhibitor can build the vector which connected the reporter gene with the lower stream of a river of (1) LOX-1 gene-expression regulatory region, can introduce (2) this vector into a host cell, can contact an examined substance to (3) this host cell, and can be chosen by detecting the manifestation of (4) reporter genes.

[0028]

LOX-1 gene-expression regulatory region can carry out cloning of LOX-1 by well-known technique from genomic DNA using the gene (for example, base sequence given in the array number 1) which carries out a code. For example, the specific approach of transcription initiation sites, such as an S1 mapping method, is well-known. Moreover, it is known by generally screening a genome cDNA library by using the base sequence of 5' end of a gene as a probe that cloning of the manifestation regulatory region will be carried out. And since the obtained clone may contain 5' untranslation region which amounts to 10 or more kbps, it can shorten or fragment by exonuclease processing etc., and only a desired part can also be obtained. About the fragment shortened or fragmented, control according to an inductor etc. in the existence of manifestation induction potency and the strength of a manifestation etc. can be considered, and the smallest unit needed for activity maintenance of LOX-1 gene-expression control can also be determined.

[0029]

Next, a reporter gene is connected so that it can operate on the lower stream of a river of the LOX-1 gene-expression regulatory region determined as mentioned above. A reporter gene is not limited especially as long as the code of the detectable polypeptide is carried out. For example, the reporter gene of common use, such as luciferase, a catalase, beta-galactosidase, and the Green fluorescence protein, can be used. The vector incorporating LOX-1 gene-expression regulatory region and a reporter gene is not limited especially that what is necessary is just what enables a reporter's manifestation in a desired host cell. Various vectors, such as a plasmid, cosmid, a virus, and a bacteriophage, can be used. In order to check the installation to the host cell of a vector, the expression vector of this invention may be carrying out the code of the gene which carries out the code of the selectable marker (for example, resistance gene to ampicillin, a tetracycline, a kanamycin, a chloramphenicol, etc.). The ligase reaction using the restriction enzyme part in a vector can perform installation of the

polynucleotide of the request to a vector easily.

[0030]

In screening of the LOX-1 manifestation inhibitor of this invention, the built expression vector is introduced next to a host cell. As a host who introduces an expression vector, the prokaryon and eukaryotic cell of the origins, such as bacteria, true fungi, vegetation, an insect, an amphibian, and the mammals, can be mentioned, and it is not limited especially. Moreover, the approach of introducing a vector to a host cell is also well-known. the case where hosts are bacteria -- a calcium chloride method and erection PORESHON -- a vector can be introduced by law etc. In addition, various approaches, such as the electric pulse terebration, the liposome method, a microinjection method, a RIPOFE cushion, RIPOFEKUTAMIN, a calcium phosphate method, and the DEAE dextran method, are well-known, and a suitable approach can be chosen and enforced according to the class of the host and expression vector which were chosen when it was this contractor.

[0031]

Next, in screening of the LOX-1 manifestation inhibitor of this invention, an examined substance is contacted to the cell containing the expression vector of this invention. Here, especially an examined substance is not limited, for example, can show quality of a natural product, such as the matter of libraries, such as a gene library, a synthetic low-molecular-weight-compound library, a synthetic peptide library, an antibody library, and a RANDAMUFA dipeptide display library, bacteria secrete, a microorganism, vegetation, an extract from the cell of an animal and a culture supernatant of these cells, and soil.

[0032]

Finally, the polypeptide which was imprinted from this gene and which was mRNA(ed) or translated detects the manifestation of a reporter gene. Although it can also detect using the matter combined with polypeptides, such as an antibody, when detecting a polypeptide, based on the activity of reporter protein, it is also detectable. For example, when luciferase, a catalase, the beta-galactosidase, etc. are adopted as a reporter gene, based on the enzyme activity of each protein, the detection approach is well-known. Moreover, when the Green fluorescence protein is used as a reporter gene, detection by carrying out direct observation of the fluorescence of the discovered protein is possible. The detection result of a reporter gene manifestation is compared by the case where an examined substance is contacted, and the case where it is not made to contact, and the matter with which the manifestation of a reporter gene is controlled by contact to an examined substance is screened.

[0033]

Moreover, it was suggested by the experiment which used the LOX-1 genetic-defect animal of this invention that LOX-1 is participating in the infectious disease of the Babesia protozoa. Therefore, if the matter which promotes the manifestation of LOX-1 in the living body can be obtained, it is useful as the remedy or prophylactic to a protozoan disease. Then, this invention offers the screening approach of a LOX-1 manifestation accelerator. In the above-mentioned reporter system, this screening approach compares the detection result of a reporter gene manifestation by the case where an examined substance is contacted, and the case where it is not made to contact, and should just screen the matter with which the manifestation of a reporter gene is promoted by contact to an examined substance.

[0034]
Moreover, an antagonist, the inhibitor or agonist of LOX-1, and the candidate compound of an accelerator can also be obtained by choosing the compound combined with LOX-1 polypeptide. The specimen expected that such screening contains the compound combined with LOX-1 polypeptide and this is made to contact, the avidity of this polypeptide and a specimen is detected, and the thing for which the compound which has the activity combined with the polypeptide of this invention is chosen is included.
[0035]
LOX-1 polypeptide used for screening may be a recombination polypeptide, or may be a polypeptide of the natural origin. Moreover, you may be a partial peptide. Moreover, you may be the gestalt which cell surface was made to discover, or a gestalt as membrane fraction. There is especially no limit as a specimen, for example, a cell extract, cell culture supernatant liquid, a fermentation micro organism production object, a marine organism extract, a plant extract, purification or a rough purification polypeptide, a nonpeptidic compound, a synthetic low molecular weight compound, and a natural compound are mentioned. LOX-1 polypeptide to which a specimen is contacted can be contacted to a specimen as membrane fraction as a gestalt made to discover on a cell membrane as a fusion polypeptide with other polypeptides as a gestalt combined with support as a meltable mold polypeptide as a refined polypeptide, for example.
[0036]
It is possible to use the approach of well-known many for this contractor as an approach of screening the polypeptide combined with this using LOX-1 polypeptide. Such screening can be performed with an immunoprecipitation method. Specifically, it can carry out as follows. DNA which carries out the code of the polypeptide of this invention -- pSV2neo pcDNA I pCD8 etc. -- the gene concerned is made to discover by an animal cell etc. by inserting in the vector for a foreign gene manifestation as the promotor who uses for a manifestation (Rigby In Williamson [(ed.)] --) SV40 early promoter GeneticEngineering Vol.3. Academic Press London p.83-141 (1982), EF-1 alpha promoter (Kim et al. Gene 91, p.217- 223 (1990)) CAGpromoter (Niwa et al. Gene 108, p.193-200 (1991)) RSV (Cullen Methods in Enzymology 152 and p.684-704 [(1987)] --) LTR promoter SRalpha promoter (Takebe et al. Mol. Cell. Biol. 8, p.466 (1988)) CMV immediate early promoter (Seed and Aruffo Proc. Natl. Acad. Sci.USA 84, p.3365-3369 (1987)), SV40 late promoter (Gheysen and Fiers J. Mol. Appl. Genet. 1, p.385-394 (1982)) Adenovirus latepromoter (Kaufman et al.Mol. Cell. Biol. 9, p. 946 (1989)) HSV TK promoter etc. -- anything may be used as long as it is the promotor who can generally use it.
[0037]
In order to make a foreign gene discover by introducing a gene into an animal cell the electroporation method (Chu, G. et al. Nucl. Acid Res. 15, 1311-1326 (1987)) calcium phosphate method (Chen, C and Okayama, H. Mol. Cell. Biol. 7, 2745-2752 (1987)) The DEAE dextran method (it Lopata(s)) M. A. et al. Nucl. Acids Res. 12 5707-5717 (1984); Sussman D. J. and Milman G. Mol. Cell. Biol. 4 1642-1643 (1985) The RIPOFE cutin method (it Derijard(s)) B. Cell 7 1025-1037 (1994); Lamb B. T. et al. Nature Genetics 5 22-30; (1993) Rabindran S.K. et al.Science 259 230-234 (1993) etc., although there is an approach It is good by any approach.
[0038]

The polypeptide of this invention can be made to discover as a fusion polypeptide which has the recognition site of a monoclonal antibody by introducing the recognition site (epitope) of a monoclonal antibody clear [singularity's] in the end of the N end of the polypeptide of this invention, or C. What is marketed as an epitope-antibody system to be used can be used (experimental medicine 13, 85-90 (1995)). The vector which can discover a fusion polypeptide with a beta galactosidase, maltose binding protein, a glutathione S-transferase, green fluorescence protein (GFP), etc. is marketed through the multi-cloning site.

[0039]

In order to make it not change the property of LOX-1 polypeptide as much as possible by making it a fusion polypeptide, only the small epitope part which consists of about ten amino acid is introduced from some, and the method of preparing a fusion polypeptide is also reported. For example, the poly histidine (His-tag), influenza agglutinin HA, Homo sapiens c-myc, FLAG, Vesicular stomatitis Virus glycoprotein (VSV-GP), T7 gene10 Protein (T7-tag), a Homo sapiens herpes simplex virus glycoprotein (HSV-tag), The monoclonal antibody which recognizes epitopes, such as E-tag (epitope on monoclonal phage), and it It can use as an epitope-antibody system for screening of the polypeptide combined with LOX-1 polypeptide (experimental medicine 13, 85-90 (1995)).

[0040]

An immune complex is made to form in immunoprecipitation by adding to the cell solution which prepared these antibodies using the suitable surfactant. This immune complex consists of LOX-1 polypeptide, a polypeptide which has it and a binding affinity, and an antibody. It is also possible to perform immunoprecipitation using the antibody to LOX-1 polypeptide besides using the antibody to the above-mentioned epitope. The antibody to LOX-1 polypeptide can refine the polypeptide which introduced into the suitable Escherichia coli expression vector the gene which carries out the code for example, of the LOX-1 polypeptide, was made to discover within Escherichia coli, and was made to discover, and can prepare it by carrying out immunity of this to a rabbit, a mouse, a rat, a goat, a fowl, etc. Moreover, it can also prepare by carrying out immunity of the partial peptide of LOX-1 compound polypeptide to the above-mentioned animal.

[0041]

For an immune complex, an antibody is Mouse IgG. It is Protein if it is an antibody. A Sepharose and Protein G It can be made to sediment using Sepharose. Moreover, it is glutathione-Sepharose when LOX-1 polypeptide is prepared as a fusion polypeptide with epitopes, such as GST. An immune complex can be made to form like the case where the antibody of LOX-1 polypeptide is used for these epitopes, such as 4B, using the matter combined specifically.

[0042]

What is necessary is just to carry out by applying correspondingly about the general approach of immunoprecipitation, for example according to an approach given in reference (Harlow, E. and Lane, D.: Antibodies, pp.511-552, Cold Spring HarborLaboratory publications, NewYork (1988)).

[0043]

SDS-PAGE is common to the analysis of a polypeptide by which immunoprecipitation was carried out, and the polypeptide combined with the molecular weight of a

polypeptide can be analyzed by using the gel of suitable concentration. Moreover, since it is difficult in this case to detect the polypeptide generally combined with LOX-1 polypeptide by the usual staining technique of polypeptides, such as Coomassie dyeing and argentation, detection sensitivity can be raised by cultivating a cell with the culture medium containing the 35S-methionine and 35S-cysteine which are radioisotope, carrying out the indicator of this intracellular polypeptide, and detecting this. If the molecular weight of a polypeptide becomes clear, the target polypeptide can be refined from direct SDS-polyacrylamide gel, and the array can also be determined.

[0044]

Moreover, it can carry out using LOX-1 polypeptide, for example, using a waist western blotting method (Skolnik, E. Y. et al., Cell (1991) 65, 83-90) as an approach of isolating the polypeptide combined with this polypeptide. Namely, the cell it is expected to be to have discovered LOX-1 polypeptide and the polypeptide to combine, The cDNA library using phage vectors (lambdagt11, ZAP, etc.) is produced from an organization. What is necessary is to make LOX-1 polypeptide and the above-mentioned filter which refined and carried out [fixed] the indicator of the polypeptide which made discover this on LB-agarose and the filter was made to discover react, and for an indicator just to detect the plaque which discovers LOX-1 polypeptide and the united polypeptide. The method of using the approach of using the antibody specifically combined with the polypeptides (for example, GST etc.) united with the approach, LOX-1 polypeptide, or LOX-1 polypeptide using the affinity of a biotin and avidin as an approach of carrying out the indicator of the LOX-1 polypeptide, the method of using radioisotope, or fluorescence etc. is mentioned.

[0045]

moreover, as other modes of the screening approach of this invention A cell Used 2-hybrid system (it Fields(es)) S. and Sternglanz, R. and Trends.Genet. (1994) 10 286-292 and Dalton S and Treisman R (1992) Characterization of SAP-1 a protein recruited by serum response factor to the c-fos serum response element.Cell 68, "597-612, MATCHMARKER Two-Hybrid System", "Mammalian MATCHMAKER Two-Hybrid AssayKit", "MATCHMAKER One-Hybrid System" (all are the Clontech make), The approach of performing using "HybriZAP Two-Hybrid Vector System" (Stratagene make) is mentioned.

[0046]

It sets to 2-hybrid system and is SRF about LOX-1 polypeptide or its partial peptide. A DNA binding field or GAL4 Make it unite with a DNA binding field, and it is made discovered in a yeast cell. From the cell it is expected to be to have discovered LOX-1 polypeptide and the polypeptide to combine A cDNA library which is discovered in the form united with VP16 or a GAL4 imprint activation field is produced. This is introduced into the above-mentioned yeast cell, and the library origin cDNA is isolated from the detected electropositive clone (if the polypeptide of this invention and the polypeptide to combine are discovered by yeast intracellular, a reporter gene is activated by both association and an electropositive clone can be checked). By making Escherichia coli introduce and discover isolated cDNA, this cDNA can obtain the polypeptide which carries out a code. It is possible to prepare the polypeptide which this combines with LOX-1 polypeptide, or its gene.

[0047]

As a reporter gene used in 2-hybrid system, although Ade[besides HIS3 gene]2 gene,

a LacZ gene, a CAT gene, a luciferase gene, PAI-1 (Plasminogen activator inhibitor type1) gene, etc. are mentioned, it is not restricted to these, for example. Screening by 2 hybrid methods can also be performed using a mammalian cell besides yeast etc. Screening of the compound combined with LOX-1 polypeptide can also be performed using an affinity chromatography. For example, LOX-1 polypeptide is fixed to the support of an affinity column, and the specimen with which having discovered the polypeptide combined with LOX-1 polypeptide here is expected is applied. As a specimen in this case, a cell extract, a cell melt, etc. are mentioned, for example. After applying a specimen, a column can be washed and the polypeptide combined with the polypeptide of this invention can be prepared.

[0048]

The obtained polypeptide can obtain DNA which carries out the code of this polypeptide by analyzing the amino acid sequence, compounding Oligo DNA based on it, and screening a cDNA library by using this DNA as a probe.

[0049]

moreover, as an approach of isolating the compound (agonist and an antagonist being included) combined with the polypeptide of not only a polypeptide but this invention To LOX-1 fixed polypeptide, for example, a synthetic compound, a natural product bank, Or a RANDAMUFA dipeptide display library is made to act. The approach of screening the molecule combined with LOX-1 polypeptide, The high throughput by the combinatorial chemistry technique The used screening approach () [Wrighton] NC; Farrell FX; Chang R; Kashyap AK; Barbone FP; Mulcahy LS;Johnson DL; Barrett RW;JolliffeLK; Dower WJ. Small peptides as potent mimetics of the protein hormoneerythropoietin, Science (UNITED STATES) Jul 26 1996 273 p458-64, Verdine GL., The combinatorial chemistry of nature. Nature (ENGLAND) Nov 7 1996 384 p11-13 and Hogan JC Jr., Directed combinatorial chemistry. Nature (ENGLAND) Nov 7 1996 384 p17-9 is well-known to this contractor.

[0050]

In this invention, the biosensor which considered as a means to detect or measure the united compound, and used the surface plasmon resonance phenomenon can also be used. The biosensor using a surface plasmon resonance phenomenon can be observed on real time as a surface plasmon resonance signal, without carrying out the indicator of the interaction between LOX-1 polypeptide and a sample compound, using the polypeptide of a minute amount (for example, BIAcore, the product made from Pharmacia). Therefore, it is possible by using biosensors, such as BIAcore, to evaluate association with LOX-1 polypeptide and a sample compound.

[0051]

Moreover, it was shown by when it became clear that that an inner-bark function rises by the deficit of LOX-1, an edema, and inflammation were controlled by the experiment which used the LOX-1 genetic-defect animal of this invention that LOX-1 antagonist and/or an inhibitor are useful as an anti-inflammatory agent in the improvement list of the symptoms to which the inner-bark function is falling. Therefore, this invention offers the remedy and anti-inflammatory agent of symptoms in which the fall of the inner-bark function which makes LOX-1 antagonist and/or an inhibitor an active principle participates. As LOX-1 antagonist and an inhibitor, a neutralizing antibody can be mentioned, for example (JP,2000-109435,A; the [international patent] WO 01/No. 64862 pamphlet).

[0052]
Here, it combines with LOX-1 specifically and a neutralizing antibody points out the antibody which makes the activity disappear or decline. The antiserum which a neutralizing antibody uses LOX-1 or its part as an antigen, and is produced by the usual technique, Polyclonal antibody () [Current Protocols] in Molecular Biology Refer to the (1987) Sons and monoclonal antibody Section () 11.12-11.13 John Wiley & [Current Protocols in Molecular] Biology (1987) Section 11.4-11.11 John Wiley It can choose from refer to the &Sons. To the neutralizing antibody of this invention, moreover, a chimeric antibody, a single strand antibody (The Pharmacology of Monoclonal Antibody, Vo. 113, Rosenburg and Moore ed., and [ ] (1994) -- 269-315 pages) Springer Refer to Verlag, Multi-singularity antibody () [LeDoussal et] al. (1992) Int. J. Cancer Supple. 7 : 58-62; Paulus (1985) Behring Inst. Mill. 78 : 118-32; Millstein and Cuello (1983) Nature 305 : 537-9; Van Dijk et al. (1989) Int. J. Cancer 43 : Refer to 944-9;WO93 / 11161 grade, A hominization antibody (Presta (1992) Curr. Op. Struct. Biol. 2: 593-6), Antibody fragments, such as Fab, Fab', and F (ab')2, Fc, Fv, are also contained in a Homo sapiens antibody (. Genet.15: WO92/03918;WO94/02602; Mendez et al. (1997) Nat refer to 146-56 grade) and a list. Even if it is furthermore embellished with the polyethylene glycol etc. if needed, they are detectable protein (the beta-galactosidase, maltose binding protein, a glutathione-S-transferase, the Green fluorescence protein, biotin, etc.) (united.). The above-mentioned antibody and above-mentioned antibody fragment which were embellished can process a neutralizing antibody with enzymes, such as a papain and a pepsin, can isolate the gene which carries out the code of combining with the desired matter if needed further, or the neutralizing antibody, and can produce it by the gene engineering-technique (refer to Borrebaeck and Larrick (1990) Therapeutic Monoclonal Antibodies and MacMillan Publishers Ltd.). Moreover, means, such as affinity columns, such as a well-known approach, for example, protein A, or Protein G, a chromatography MATOGURA fee column, a filter, an ultrafiltration, a salting-out, and dialysis, can refine an above-mentioned antibody suitably like purification of other protein if needed (Antibodies: refer to [A Laboratory Manual., Harlow, and] David Lane ed. (1988) Cold Spring Harbor Laboratory).
[0053]
As a disease in which the fall of an inner-bark function participated, hyperlipidemia, diabetes mellitus, hypertension, an ischemic disease, etc. are illustrated. An ischemic disease includes the ischemic disease by vascular injury, a thrombus, infarction, arteriosclerosis, the artery plug, artery lock out, an aneurysm, etc.
[0054]
The antagonist and/or inhibitors of LOX-1, such as an antibody, can be prepared combining support, a medium, etc. which are suitably permitted pharmacologically as the constituent for a therapy, or an anti-inflammatory agent in an above-mentioned LOX-1 manifestation inhibitor and a list. For example, a stabilizer, anti-oxidants (ascorbic acid etc.), a surfactant, buffers (a citric acid, a phosphoric acid, other organic acids, etc.), a chelating agent, corrigent, a binder, perfume, a physiological saline, an isotonic solution, an excipient, antiseptics, a solubilizing agent, etc. can be blended. Moreover, amino acid, such as an asparagine, an arginine, a glycine, a glutamine, and a lysine, serum albumin, gelatin, a carbohydrate, protein, a low-molecular-weight polypeptide, sugar-alcohol (a mannitol, sorbitol, etc.), a saccharide (a polysaccharide and monosaccharide), an immunoglobulin, etc. may also be included as other matter if needed. When considering

as the water solution for injection, it is desirable to mix with the isotonic solution (for example, thing containing D-sorbitol, D-mannose, D-mannitol, and a sodium chloride) containing the adjuvant of a physiological saline, grape sugar, or others, and it may use together with a still more suitable solubilizing agent (ethanol etc.), for example, alcohol, polyalcohols (propylene GUKORU, PEG, etc.), a nonionic surfactant (polysorbate 80, HCO-50 grade), etc.

[0055]

Moreover, the antagonist and/or inhibitors of LOX-1, such as an antibody, can be enclosed with a microcapsule at a LOX-1 manifestation inhibitor and a list if needed. As a microcapsule, what consists of a hydroxymethyl cellulose, gelatin, Pori [methyl methacrylic-acid], etc., for example is well-known. Moreover, the colloid drug delivery system (refer to Remington's Pharmaceutical Scinece 16th ed. and Oslo ed. (1980)) using liposome, an albumin microsphere, microemulsion, a nano particle, a nano capsule, etc. is also available. In addition, the approach of preparing as sustained-release drugs is also well-known (for example). Langer et al. (1981) J. Biomed. Mater. Res. 15 : 167-277; Langer (1982) Chem. Tech. 12 : 98-105; Sidman et al. (1983) Biopolymers 22 : 547-56; US3,773,919; EP58,481; It is referring to the EP133,988 grade. It can apply to the constituent and drugs of this invention. Moreover, it is also possible to perform a nest and gene therapy for the gene which carries out the code of the antibody to LOX-1 to the well-known vector for gene therapies.

[0056]

Administration to a patient is preferably performed by injection and intravenous drip, and intraarterial injection, an intravenous injection, and subcutaneous injection are mentioned. In addition, it is also possible to pass and to prescribe a medicine for the patient according to paths, such as transderma and taking orally, in a bronchial tube and a muscle in a nasal cavity. Although a dose changes with classes, routes of administration, etc. of the class of a patient's weight and age, and illness with which it deals and a symptom, and the drugs to be used, if it is this contractor, it can choose the suitable dose according to each situation suitably.

[0057]

[Example]

Hereafter, although an example explains this invention concretely, this invention is not restricted to these examples.

[Example 1] Production of the vector for homologous recombination of LOX-1 gene

In this example, in order to build the vector for homologous recombination of mouse LOX-1 gene, cloning of a mouse LOX-1 genome gene was performed first. In order to perform a positive/negative selection according to Mansour's and others report (Nature 336: 348 (1988)) using this genomic DNA, the vector for homologous recombination which inserted the neomycin resistance gene and the thymidine kinase gene was built. Under the present circumstances, a neomycin resistance gene is inserted so that a part of 7 and 8 [a part of exon 6 and] may be replaced, and it prevented from producing normal LOX-1. It explains concretely below.

[0058]

A. Cloning of mouse LOX-1 gene

SUPERSCRIPT Choice System (GIBCO BRL) It uses, the cDNA library (Lambda gt10) of a mouse (C57BL/6) placenta is produced, and it is cow LOX-1. Plaque hybridization was performed by using all the open reading frames of cDNA as a probe. As a result, 15

electropositive clones were obtained. Among those, sequencing of the clone T is carried out and it is mouse LOX-1. All the open reading frame arrays of cDNA were determined. In this way, mouse LOX-1 obtained The array of cDNA is shown in the array number 1, and the amino acid sequence is shown in the array number 2.
Cloning of the mouse LOX-1 genomic DNA was carried out as follows. First, mouse LOX-1 It is Mouse (129-/SV) genomic by the plaque hybridization method, using all the open reading frames of cDNA as a probe. DNA Lambda FIX II library was screened. It is pBluescriptII, respectively about the insert of 3kbp(s), 8kbp, and 5kbp which might be cut with the restriction enzyme SacI in the phage DNA prepared from the obtained electropositive clone D. Subcloning was carried out to the SacI site of SK (-) (clones D3, D8, and D5). Then, sequence of the base sequence of clones D3, D8, and D5 was performed, and the exon 4 checked that the first half of exons 5-8 was contained in D8, and the second half of an exon 8 was contained in D5 at D3 (the base sequence of exons 3-8 is respectively shown in the array numbers 3-10). Main restriction enzymes (EcoRV, BamHI, ApaI, HinDIII) cut the field of LOX-1 gene covered by Clone D, and the map of the location of the restriction enzyme site was produced (drawing 1).
[0059]
B. Construction of the vector for homologous recombination
It included in the EcoRI site of pGEM-Neor which flush-end-ized the fragment of about 3.8 kbp(s) which cut the mouse LOX-1 gene fragment contained in a clone D8 by ClaI and ApaI, and were produced by T-four polymerase, and was flush-end-ized by T-four polymerase (p5LOXNeo). It is pBluescriptII about the thing of about 2 kbp(s) among the fragments which cut the mouse LOX-1 gene fragment contained in a clone D5, and can do it in KpnI and BamHI on the other hand. It incorporated between the KpnI site of SK (-), and the BamHI site. After cutting down this gene fragment by Asp718I and BamHI and flush-end-izing by T-four polymerase, it included in pMC1-tk flush-end-ized by T-four polymerase (pLOX3kt). The fragment which cut the obtained clone by SalI and XhoI and was cut down was built into the SalI site of p5LOXNeo, and the vector for homologous recombination (LOX-1 targeting Vector) was obtained. This vector has the following descriptions (drawing 1).
(i) The neomycin resistance gene is inserted in the form where it replaces from the second half of the 6th exon in the first half of the 8th exon.
(ii) It has a thymidine kinase gene as a marker gene for negative selection. (iii) The upstream of a neomycin resistance gene is [about 3.8 kbs and the neomycin resistance gene lower streams of rivers of the analogous segment with wild type LOX-1 gene] about 2 kbs.
[0060]
[Example 2] ES with variation LOX-1 gene by homologous recombination Establishment of a cell
Embryonic stem cell obtained from the 129-/SvJ mouse by the electroporation method in the vector for homologous recombination in this example It introduced into RW-4 and, subsequently G418 performed selective culture. About obtained G418 resistance colony, the homologous recombination object was authorized by Southern blotting. Hereafter, it explains concretely.
Vector DNA for homologous recombination (LOX-1 Targeting Vector) By cutting 25-30microg by XhoI, it line-ized and refined. It is this DNA Mouse embryonic stem cell RW-4 and 3x106 It suspends in the buffer solution for electroporation containing an

individual (20mM HEPES pH7.0, 137mM NaCl, 5mM KCl, 6mM D-glucose, 0.7mM Na2HPO4), and is Field. Strength 185 V/cm, Capacitance Transgenics was performed on 500-micro F conditions. Selective culture was performed from 24 hours after installation by G418 [with a final concentration of 200microg //ml] (Geniticin) (SIGMA company G-9516). In culture of an embryonic stem cell, at the Dulbecco alteration Eagle's-medium (DMEM) (Gibco/BRL 11965-084) culture medium Fetal calf serum of 15% of final concentration (Hyclone SH30071), The L-glutamine of final concentration 2mM (Gibco/BRL 25030-081), The nonessential amino acid whose final concentration is 100microM, respectively (Gibco/BRL 11140-050), HEPES of final concentration 10mM (Gibco/BRL 15630-080), The penicillin/streptomycin whose final concentration is 100U/ml, respectively (Gibco/BRL 15140-122), beta-mercaptoethanol (SIGMA company M-7522) with a final concentration [M] of 100micro and the thing which added final concentration 1000U/ml ESGRO (LIF) (Gibco/BRL 13275-029) were used (it is described as ES culture medium below).

[0061]

moreover, as a feeder cell for embryonic stem cells The MEF (Mouse Embryonic Fibroblast) cell isolated from the germ of E14.5 is used. Culture medium to DMEM (Gibco/BRL 11965-084) culture medium Fetal calf serum of 10% of final concentration (Hyclone SH30071), The L-glutamine of final concentration 2mM (Gibco/BRL 25030-081), The nonessential amino acid whose final concentration is 100microM, respectively (Gibco/BRL 11140-050), What added the penicillin/streptomycin (Gibco/BRL 15140-122) whose final concentration is 100U/ml, respectively was used (it is described as a MEF culture medium below). The MEF cell which even confluence was made to cultivate with the flask of 2 150cm is stripped by the trypsin/EDTA (0.05%/1mM and Gibco/BRL 25300-047), and it rescattered by optimum respectively concentration in four 10cm dish and two 24 hole plates, two 6 hole plates, six 25cm two flasks, and two 75cm two flasks.

[0062]

The passage of the G418 resistance colony which is the following, made and appeared from the 5th after transgenics was carried out to the plate of 24 holes. That is, after moving and changing G418 resistance colony to the microplate of 96 holes containing the trypsin / EDTA solution of 150microl using the pipet man of P-200 and processing within a 37-degree C incubator for 20 minutes, it was made the single cell by carrying out pipetting by the pipet man of P-1000. This cell suspension was moved to the plate of 24 holes, and substitute culture was continued. The cell on the plate of 24 holes was divided into two, the object for cryopreservation, and the object for a DNA extract, two days after. That is, a trypsin/EDTA was 500microl Added to the cell, and it processed within the 37-degree C incubator for 20 minutes, and it was made the single cell, when ES culture medium was 500microl Added and carried out pipetting calmly by the pipet man of P-1000. Then, the one half of cell suspension was moved to 24 hole plate containing 1ml ES culture medium, and 1ml of ES culture media was added also to the original 24 hole plate. After extracting the culture medium of one of the two's 24 hole plate two days after to the pan, and it put 1ml of culture media for freezing which added the fetal calf serum whose final concentration is 10%, and the dimethyl sulfoxide (DMSO) (Sigma D-5879) whose final concentration is 10% into ES culture medium and they carried out the seal to it, cryopreservation was carried out at -70 degrees C.

[0063]

Assay of a homologous recombination object went as follows by Southern blotting. That is, after removing a culture medium from each well of 24 hole plate which the cell increased to the confluent condition and washing by PBS, they are 250microl and proteinase about a dissolution buffer (1% SDS, 20mM EDTA, 20mM Tris pH7.5). K(20mg/(ml)) 5microl could be added, and it warmed at a swing and 52 degrees C for 1 to 12 hours. The phenol/chloroform extraction extracted DNA from the dissolved sample, and it used as template DNA for Southern blotting.

[0064]

Southern blotting performed Southern hybridization by using as a probe the array between the EcoRV-SacI sites of the exon 5 upstream made to suffer a loss by making it permute by the neomycin resistance gene, and the array between the XbaI-ApaI sites of exon 8 lower stream of a river. The number of the clones considered to be a homologous recombination object from this result was two among 368 investigated G418 resistance clones (a clone 116, clone 244). (Drawing 2)

[0065]

The clone from which homologous recombination was checked by southern blot analysis was dissolved by warming 96 hole plate which had carried out cryopreservation at 37 degrees C, and carried out the passage to 24 hole plate. Culture media were exchanged in order to remove DMSO and a liquid paraffin for this 24 hole plate after culture at 37 degrees C for 24 hours. When each clone reached confluence 75 to 90%, the passage was carried out to 6 hole plate from 24 holes. furthermore, the thing which carried out until [confluent] growth 75 to 90% at 6 hole plate -- 2 -- ****** -- having had -- cryopreservation of one hole was carried out and the one remaining holes were used for the impregnation to a blastocyst, and a DNA extract in the place.

[0066]

Cryopreservation was performed as the following. That is, it is ESQ about a cell. 0.5ml ESQ after carrying out a rinse twice by PBS After adding Trypsin (LEXICON company The Mouse Kit), keeping it warm for 15 - 20 minutes at 37 degrees C and performing trypsinization, the 0.5 moreml embryonic stem cell culture medium was added, pipetting was performed 35 to 40 times, and the lump of an embryonic stem cell was made to dissociate completely. This cell suspension was moved to 15ml centrifugal tube, the well was washed by the 1 moreml embryonic stem cell culture medium, and it collected in the tube. The at-long-intervals alignment of the tube is carried out by 1,000rpm for 7 minutes, and a culture medium is removed. 0.25ml It re-suspends in an embryonic stem cell culture medium, and is 2 [0.25ml]. x freezing culture medium was added. Moved the contents of the well to the KURAIOJIE nick vial, and it was made to freeze at -80 degrees C, and saved in liquid nitrogen.

[0067]

Cell the impregnation to a blastocyst, and for a DNA extract After making the lump of an embryonic stem cell dissociate completely, the quarter was used for the impregnation to a blastocyst, and the passage was carried out to the third of the remaining cells, and 60mm dish which carried out the gelatin coat of 2/3, respectively. The former extracted the genomic DNA for southern blot analyses in the place which the cell increased even to confluence, and the latter cell was divided into three and frozen in the place increased even to confluence.

[0068]

[Example 3] Production of the chimeric mouse by the embryonic stem cell with

recombination LOX-1 gene
ES by which homologous recombination was checked About the cell clone, the chimera germ was produced by having made the blastocyst of C57BL / 6J system mouse into the host germ, it was transplanted to the uterine horn of a pseudopregnancy mouse, and offspring was obtained. Extraction of a host germ is 100microM on the 2nd day of pregnancy. EDTA was added. It is a Whitten's culture medium and carried out by flowing in an oviduct and a uterus. Eight cell term germ or morula was cultivated by the Whitten's culture medium for 24 hours, and the obtained blastocyst was used for impregnation. ES used for impregnation It was put at 4 degrees C until it made TE processing distribute on 2 or the 3rd and presented micromanipulation with it, after carrying out the passage of the cell. ES As a pipet for impregnation of a cell, it is Cook. polar made from IVF body extrusion pipette (bore of about 20 micrometers) was used. After extending thinly a minute glass tube (NARISHIGE) with an outer diameter of 1mm, using a microelectrode production machine (Sutter P-98/IVF) as a pipet for germ retention, it cut in the part with an outer diameter of 50-100 micrometers using micro FOJI (De Fonburun), and what processed aperture into 10-20 micrometers further was used. The pipet for impregnation and the pipet for retention bent about 5mm part about 30 degrees from the tip, and connected it to the micromanipulator (LEITZ). As a chamber used for micromanipulation, what pasted up the cover glass on the hole vacancy slide glass by beeswax is used, and it is 0.3% of about 20microl on it. Hepes-buffered which added BSA The drop of a Whitten's culture medium was covered by every two pieces, and the top face was covered with the liquid paraffin (Nakarai Tesuku 261-37 SP). About 100 embryonic stem cells are put into one drop, 10-15 extended blastocysts are put into another side, and they are 10-15 ES(s) per germ. The cell was poured in. All micromanipulation was performed under the inverted microscope. The actuation germ was transplanted to the uterine horn of a day [of pseudopregnancy / 2nd] ICR system acceptance female after culture of 1 - 2 hours. The cesarean section was performed and the foster parent was made to nurse about the acceptance female which did not carry out the extraction of the offspring even if it continued till the calculated date of confinement.
[0069]
To 40 blastocysts of C57BL / 6J system mouse, it is a clone 116. As a result of pouring in an embryonic stem cell, the impregnation to 40 blastocysts was successful (100% of success percentage). As a result of transplanting these 40 pieces to the uterine horn of a day [of pseudopregnancy / 2nd] ICR system acceptance female, the offspring of 14 animals was obtained. The color of hair of the part originating in a homologous recombination object presents a wildness color, and the color of hair of the part originating in C57BL / 6J system mouse presents a black color. One in the obtained offspring could judge with the chimeric mouse from the color of hair, and the male was shown in gestalt. The contribution of an embryonic stem cell judged from the color of hair in this chimeric mouse was 95%.
[0070]
[Example 4] Assay of the transfer to the germ cell line of a homologous recombination object
The chimeric mouse of an example 3 was made to cross with C57BL / 6J system mouse, and it authorized whether the offspring of the embryonic stem cell origin would be obtained. The color of hair of the offspring by which the extraction will be carried

out if the reproductive cell of a chimeric mouse originates in the embryonic stem cell presents a wildness color, and if it originates in the blastocyst of C57BL / 6J system mouse, it will present black. The mouse of a wildness color was born by mating and the transfer to the germ cell line of an embryonic stem cell was checked.

[0071]

Next, DNA was extracted from a part of tail of these wildness color mice, and it investigated whether variation LOX-1 would be transmitted by Southern blotting. Consequently, it was checked that variation LOX-1 allyl compound is transmitted in the offspring of the clone 116 embryonic-stem-cell origin.

[0072]

By mating of hetero deficit mice which has variation in LOX-1 gene of the allyl compound of one side, the gay deficit mouse which has variation in the allyl compound of both sides was produced. Southern blotting performed for a wild type, a hetero deficit, and the analysis of each genotype of a gay deficit mouse. Existence of a wild type allyl compound could be detected and the case where the wild type mouse had what cannot detect existence of a variation allyl compound detected, and both a gay deficit mouse, and a wild type and a variation allyl compound were detected in the thing which could not detect existence of a wild type allyl compound on the contrary, but has detected existence of a variation allyl compound was judged to be a hetero deficit mouse (drawing 3).

The ratio of a wild type:hetero deficit:gay deficit mouse of each genotype of the offspring obtained as a result was the rate of 1:2:1 mostly as Mendel's laws.

[0073]

[Example 5] Manifestation analysis of LOX-1 in a LOX-1 deficit mouse

A thorax main artery is extracted from a LOX-1 deficit mouse, and they are after freezing and Polytron at liquid nitrogen. PT Ize [HOMOJIE] is carried out in 1200 and it is TRIzol. Reagent (Invitrogen) It uses. The total RNA was extracted. The procedure with the same said of a wild type (C57BL/6) extracted the total RNA. A reverse transcription reaction is Superscript. Firststrand Synthesis system for RT-PCR (Invitrogen) It carried out by using. ** which used KISAMA to random as a primer of a reverse transcription reaction, and was extracted from the thorax main artery RNA It is SuperscrptII, using 1ug as a template. The reverse transcription reaction was performed in 50U, and cDNA was obtained.

[0074]

It is Platinum in 5% of the produced reverse transcription reactant. Taq DNA polymerase (Invitrogen) It was used for PCR by 0.5U. Mouse LOX-1 In cDNA magnification, it is a sense primer. : 5'-aggtccttgtccacaagactgg-3' (array number: 11), antisense primer: 5'-acgcccctggtcttaaagaattg-3' (array number: 12) is used, and it is 94 degrees C. 40 seconds 57 degrees C 1 minute 72 degrees C 35 cycle reactions were performed on the conditions for 1 minute. It is GAPDH as an internal standard of the amount amendment of samples to coincidence. PCR of cDNA was performed. Sense primer: 5'- gaccacagtccatgacatcact -3' (array number: 13), antisense primer: 5'- tccaccaccctgttgctgtag -3' (array number: 14) is used, and it is 94 degrees C. 40 seconds 60 degrees C 1 minute 72 degrees C 25 cycle reactions were performed on the conditions for 1 minute. Electrophoresis of the magnification product was carried out by 4% agarose gel containing the ethidium bromide, the mouse LOX-1 magnification product of 273bp and the mouse GAPDH magnification product of 453bp(s) were

visualized with UV lamp, and a photograph was taken with the CCD camera.
[0075]
Consequently, it sets to a wild type (C57BL/6), and is mouse LOX-1 of 273bp. Although the cDNA specific PCR product was checked, it did not accept in the LOX-1 deficit mouse. In addition, the band strength on the electrophoresis of the magnification product of GAPDH used as an internal standard is LOX-1. It was almost equivalent when a knockout mouse was compared with a contrast mouse. Therefore, to be sure, it is LOX-1 with a LOX-1 deficit mouse. It was checked that the manifestation of mRNA has disappeared. (Drawing 4) .
[0076]
[Example 6] Examination of the vascular endothelial cell dependency relaxation response
1. Adjustment of Solution
The blood vessel taken out from the mouse experimented in the Krebs solution (118mM NaCl, 4.7mM KCl, 1.2mM MgSO4, 1.2mM NaH2PO4, 2.5mM CaCl2, 25mM NaHCO3, 10mM glucose). : which adjusted the high potassium solution of the following presentation — 62.7mM NaCl 60mM KCl 1.2mM MgSO4 1.2mM NaH2PO4 2.5mM CaCl2 25mM NaHCO3 10mM Glucose. The Krebs solution and the high potassium solution performed bubbling by CO2 95% O 2 or 5%.
[0077]
2. Production of Ring Sample of Blood Vessel
The thorax of the mouse of a 10-weeks old male was opened, the thorax main artery was taken out the whole heart, and it put into the petri dish into which the Krebs solution was put. Under the microscope, the main artery was separated from the heart and the fat tissue and connective tissue which reach the aortic perimeter were separated so that an organization might not be pulled. And the main artery ring sample of 1mm of **** was taken out from between the tee of a subclavian artery, and the tees of a posterior intercostal artery among main arteries, and it was used for the experiment.
[0078]
3. Experimental Device
The main artery ring sample was covered over the hook of two attached into the chamber which put in the 5ml Krebs solution. One in the hook of two is being fixed to equipment, and one more was connected with the isometric transducer (UC-5A UFER) which senses aortic contraction and relaxation as a pressure concerning a hook, and is changed into an electrical signal. The electrical signal from tolan SUYUSA was amplified with amplifier (PAS-401 Star Medical), and was recorded. Record is PowerLab. System (ADInstrument) It was used.
[0079]
A. The check of inner-bark dependency relaxation
By lengthening spacing of the hook of two concerning a main artery ring sample, the pressure of 0.5g was put on the blood vessel. When the pressure was put on the blood vessel, since the main artery loosened to return, it applied ** again and made it resting tension until ** concerning a main artery became fixed by 0.5g. Since standing was carried out for 90 minutes after resting tension is stabilized, the solution of a chamber was replaced with the high potassium solution, and high potassium solution induction contraction was made to cause. When this contraction was stabilized, the solution of a

chamber was returned to the Krebs solution and the blood vessel was loosened to resting tension. Since contraction by this high potassium solution induction is repeated 3 times and carried out standing for 30 minutes, it was used for measurement. In order to evaluate relaxation of the inner-bark dependency of the main artery ring sample of a mouse, the blood vessel was shrunk first. PGF2a (WAKO) was used as vasoexcitor material. After the contraction obtained with the contraction matter was stabilized, relaxation of an inner-bark dependency was seen by acetylcholine (Ovisot notes Daiichi Pharmaceutical). After making L-NAME (Wako Pure Chem) which is NO composition inhibitor act on that make acetylcholine act on the blood vessel which removed the inner bark by letting yarn pass to a blood vessel lumen, and relaxation does not occur in order that relaxation by this acetylcholine may confirm whether it is relaxation of an inner-bark dependency, and the blood vessel which left the inner bark, it was made to contract with the contraction matter, and it checked that relaxation by acetylcholine did not occur. In a detail, it is ** about an inner bark from the (drawing 5 upper case). Although relaxation occurred in the main artery of the wild type mouse carried out (left of the drawing 5 upper case), relaxation was suppressed through the blood vessel which relaxation was suppressed when L-NAME which is NO generating inhibitor was made to act (inside of the drawing 5 upper case), and removed the inner bark (right of the drawing 5 upper case). Therefore, it was shown that relaxation of a vascular endothelial cell dependency has occurred by administration of acetylcholine. The concentration of the acetylcholine which prescribed the figure on a chart for the patient, and was made to act is expressed, and it is shown that "8" was set to 10-8M and "7" was set to 10-7M. It is 3x10-8M between "8" and "7", and the degree of "7" shows having made the acetylcholine of 3x10-7M act. the cable address on a chart "SNAP" -- NO generating -- agent S-nitroso-N-acetyl-DL-penicillamine (S-Nitroso-N-acetyl-DL-penicillamine) and "L-NAME" show NO generating inhibitor NG-nitro-L arginine methyl ester hydro chloride (NG-Nitro-L-Arginine Methyl EsterHydrochloride).

[0080]

B. The inner-bark dependency of a wild type mouse and a LOX-1 genetic-defect mouse

The comparison of relaxation of the inner-bark dependency of a wild type mouse and a LOX-1 genetic-defect mouse applied resting tension first as above-mentioned, and since contraction by the high potassium solution is performed 3 times and carried out standing for 30 minutes, it was performed. Since the blood vessel was first shrunk by PGF2a, as change of the tension concerning a blood vessel, it measured and acetylcholine compared relaxation by emission of NO from an inner bark. You flushed the drug in a chamber and made it stabilized for 30 minutes by changing the Krebs solution in a chamber several times after that. oxLDL was added after that, it was made to contract with the contraction matter, and acetylcholine measured and compared relaxation of the inner-bark dependency in the condition of having made oxLDL acting. (Drawing 5 the middle, the lower berth)

[0081]

Consequently, although relaxation decreased with the wild type mouse after the oxLDL operation when the inner-bark dependency relaxation by acetylcholine was compared before and after making oxLDL act, in the knockout mouse, a difference was not seen in order. (WT; drawing 5 the middle, KO; drawing 5 lower berth) . If the quantum of

extent of relaxation by acetylcholine is respectively carried out by the wild type and the knockout mouse after making oxLDL act Contraction by PGF2a is set to 0, and the base line is set to 100. Acetylcholine concentration In 10-8M, respectively 1.50**0.94 and 7.62**4.33, In 3x10-8M, it is 37.74**3.35 and 68.41**3.78 1.67**0.71, 32.76**5.77, and 10-7M at the time of 18.35**3.27, 53.90**2.65, and 3x10-7M. The significant difference was looked at by relaxation by the acetylcholine of a wild type mouse and a knockout mouse.

[0082]

[Example 7] Effect of Lox-1 deficit in protozoal infection

A. Babesia rodhaini and Babesia Maintenance of microti

Babesia rodhaini and Babesia microti was maintained by the blood passage (bloodpassage) in the ICR mouse. First, the freezing lytic infection erythrocyte (frozen-thawed infected red blood cells; IRBC) was inoculated into mouse intraperitoneal. The IRBC number in the blood smear which carried out the Giemsa stain determined the rate of PARASHITEMIA. The mouse was killed and blood was collected, after reaching PARASHITEMIA 50%.

[0083]

B. Babesia microti infection

It carried out the peritoneum inscribed kind of the 1x107IRBC at a time to a 8-weeks old Lox-1 knock-out female mouse and C57BL / 6J female mouse, and each four groups. It used three more animals at a time as non-infected control about each group. The rate of PARASHITEMIA, weight, and hematocrits were collected. The IRBC number in the blood sample of the tail which carried out the Giemsa stain determined the rate of PARASHITEMIA. The hematocrit was measured with the micro haematocrite method. About the rate of PARASHITEMIA, a decision was made and the hematocrit was measured day by day [2] after infection every day. Consequently, Babesia to a Lox-1 deficit mouse microti (1 x 107 IRBC) In infection, changing to 3 **** was observed with the Lox-1 deficit mouse to the peak of bimodal PARASHITEMIA being shown with the wild type mouse. In addition A difference with a wild type mouse was not accepted by transition of a hematocrit value. (Drawing 6)

[0084]

C. Babesia rodohaini infection

It is [a 8-weeks old Lox-1 knock-out female and C57BL / 6J female mouse, and] 1 at a time to each six groups. x Intraperitoneal inoculation of the 104IRBC is carried out, and it is Babesia. They are PARASHITEMIA and HEMATOKU like the case of microti. The Ritt value was measured. Consequently, Babesia to a Lox-1 deficit mouse Although the difference was not accepted in the survival rate after rodhaini (10,000 IRBC) infection between wild type mice, a significant reduction of the inclination to go up an early stage and more quickly than that of PARASHITEMIA, and a hematocrit value was observed. (Drawing 7)

[0085]

As mentioned above, in the infection experiment of the Babesia protozoa which are parasitic on an erythrocyte like malaria, the results which suggest the intervention to this protozoan disease of Lox-1 were acquired. Although it is known that Lox-1 will make an aging erythrocyte ligand, the detail of the function to the Babesia infection erythrocyte is unknown.

[0086]

[Example 8] Examination of an acceptor reaction to a carrageenin guide-peg edema
The 6-8-weeks old Lox-1 knock-out male mouse and the normal mouse were used for the experiment. The animal was held in the breeding room of lighting (7:00 - 19:00) for 12 hours on the room temperature of 20-26 degrees C, 40 - 70% of humidity, and the 1st, and a commercial cubed diet (F-2, Funabashi farm) and tap water were made to take in freely, and it bred them. In addition, the animal presented the carrageenin guide-peg edema method with the good thing of general status, after carrying out preliminary breeding the 3rd day or more.
[0087]
Hypodermically [right-hand side hind-foot planta] was injected with the 1%lambda-carrageenin (Lot No. P-13, Zushi chemistry) of 0.05mL(s) dissolved in the physiological saline (Otsuka Pharmaceutical works), using the mouse (weights 14.0-20.0g) made to abstain from food overnight one groups [eight]. the 0. -- the guide-peg volume was measured 5, 1, and 3 hours after, and the rate of an edema (%) was computed from the value before prophlogistic agent administration (Table 1, drawing 8).
[0088]
[Table 1]

時間 (hr)	0	0.5	1	3
WT	0	45.6	44	64.6
		6.4	7.7	5.7
KO	0	13.8	28.8	48.1
		3.4	4.8	5

[0089]
An average ** standard error shows results. In the case of the carrageenin guide-peg edema method, it authorized dispersibility that 2 assay of a normal mouse group and a knockout-mouse group between groups is based on Bartlett (or F test) etc., and performed the t test of Student. consequently, carrageenin injection 0. in a normal mouse -- the rates of an edema of 5, 1, and 3 hours after were 45.6**6.4, 44.0**7.7, and 64.6**5.7%, respectively. On the other hand, the rate of an edema in a knockout mouse was low compared with 13.8**3.4, 28.8**4.8 and 48.1**5.0%, and a normal mouse respectively, and the significant decline in the rate of an edema was observed especially carrageenin injection 0.5 and 3 hours after.
[0090]
Moreover, after guide-peg volume measurement 3 hours after, the animal was slaughtered by cervical-vertebra dislocation and the guide-peg root of a prophlogistic agent administration guide peg was fixed with neutral-buffered-formalin liquid 10%. after immobilization and formic-acid deashing -- carrying out -- a law -- according to the

method, the hematoxylin-and-eosin (HE) stained specimen was produced, and pathology histological inspection was carried out (Table 2, drawing 9).

[0091]

[Table 2] The pathology histological view of a mouse guide-peg root hypodermically

処置	カラゲニン							
使用動物	正常マウス				ノックアウトマウス			
動物数	8				8			
程度	−	+	++	+++	−	+	++	+++
皮下組織/筋層の浮腫			7	1		4	4	
皮下組織/筋層の炎症性細胞浸潤		1	7			7	1	
皮下組織の出血		8			1	6	1	

Change extent; it is +++:altitude −:change nothing, slightly [+:], and whenever [middle / of ++:].

[0092]

Consequently, as a view common to a normal mouse, inflammatory cell infiltration continued a little broadly, and was observed by the edema list of whenever [middle] in the subcutaneous tissue and tunica muscularis of a guide-peg root, and bleeding also appeared here and there further. On the other hand, also in the knockout mouse, extent of inflammatory cell infiltration was mitigated compared with the normal mouse in the edema list homogeneous as a normal mouse especially in subcutaneous tissue and the tunica muscularis although **** observation was carried out, and each inflammatory response in seven examples was slight among eight examples.

As mentioned above, as a result of considering the operation over the carrageenin guide-peg edema in a knockout mouse and a normal mouse, it saw from the rate of an edema, and the pathology organization view, and control of an inflammatory response was suggested compared with the normal mouse in the knockout mouse.

[0093]

[Effect of the Invention]

In the analysis using the LOX-1 genetic-defect mouse produced this time, LOX-1 antagonist became clear [that it is useful to the improvement of the symptoms to which the inner-bark function is falling]. Moreover, it became clear that LOX-1 is involving also in infection of protozoa. Furthermore, with the LOX-1 genetic-defect mouse, the depressant action of an inflammatory response was accepted and relation with the inflammatory response of LOX-1 was suggested. Thus, the LOX-1 genetic-defect animal of this invention is a useful model animal, in order to clarify the new function of LOX-1. Moreover, the mouse with which the function of LOX-1 gene of this invention was knocked out turns into a useful model mouse, when exploring the role

of the onset of arteriosclerosis, progress, and other LOX-1 strange genes.
[0094]
[Layout Table]

SEQUENCE LISTING

(110) CHUGAI SEIYAKU KABUSHIKI KAISHA
NATIONAL CARDIOVASCULAR CENTER RESEARCH INSTITUTE

〈120〉 LOX-1 gene deleted animal

〈130〉 C1-A0234

〈160〉 7

〈170〉 PatentIn Ver. 2.1

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〈212〉 DNA
〈213〉 Murinae gen. sp.

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〈210〉 2
〈211〉 363
〈212〉 PRT
〈213〉 Murinae gen. sp.

〈400〉 2

Met	Thr	Phe	Asp	Asp	Lys	Met	Lys	Pro	Ala	Asn	Asp	Glu	Pro	Asp	Glu
1					5				10					15	

Lys	Ser	Cys	Gly	Lys	Lys	Pro	Lys	Gly	Leu	His	Leu	Leu	Ser	Ser	Pro
			20					25					30		

Trp Trp Phe Pro Ala Ala Met Thr Leu Val Ile Leu Cys Leu Val Leu
35 40 45

Ser Val Thr Leu Ile Val Gln Trp Thr Gln Leu Arg Gln Val Ser Asp
50 55 60

Leu Leu Lys Gln Tyr Gln Ala Asn Leu Thr Gln Gln Asp Arg Ile Leu
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Glu Gly Gln Met Leu Ala Gln Gln Lys Ala Glu Asn Thr Ser Gln Glu
85 90 95

Ser Lys Lys Glu Leu Lys Gly Lys Ile Asp Thr Leu Thr Gln Lys Leu
100 105 110

Asn Glu Lys Ser Lys Glu Gln Glu Glu Leu Leu Gln Lys Asn Gln Asn
115 120 125

Leu Gln Glu Ala Leu Gln Arg Ala Ala Asn Ser Ser Glu Glu Ser Gln
130 135 140

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145 150 155 160

Lys Ser Lys Glu Gln Glu Glu Leu Leu Gln Met Ile Gln Asn Leu Gln
165 170 175

Glu Ala Leu Gln Arg Ala Ala Asn Ser Ser Glu Glu Ser Gln Arg Glu
180 185 190

Leu Lys Gly Lys Ile Asp Thr Leu Thr Leu Lys Leu Asn Glu Lys Ser
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Lys Glu Gln Glu Glu Leu Leu Gln Lys Asn Gln Asn Leu Gln Glu Ala
210 215 220

Leu Gln Arg Ala Ala Asn Phe Ser Gly Pro Cys Pro Gln Asp Trp Leu
225 230 235 240

Trp His Lys Glu Asn Cys Tyr Leu Phe His Gly Pro Phe Ser Trp Glu
245 250 255

Lys Asn Arg Gln Thr Cys Gln Ser Leu Gly Gly Gln Leu Leu Gln Ile
260 265 270

Asn Gly Ala Asp Asp Leu Thr Phe Ile Leu Gln Ala Ile Ser His Thr
275 280 285

Thr Ser Pro Phe Trp Ile Gly Leu His Arg Lys Lys Pro Gly Gln Pro
290 295 300

Trp Leu Trp Glu Asn Gly Thr Pro Leu Asn Phe Gln Phe Phe Lys Thr
305 310 315 320

Arg Gly Val Ser Leu Gln Leu Tyr Ser Ser Gly Asn Cys Ala Tyr Leu
325 330 335

Gln Asp Gly Ala Val Phe Ala Glu Asn Cys Ile Leu Ile Ala Phe Ser
340 345 350

Ile Cys Gln Lys Lys Thr Asn His Leu Gln Ile
355 360

〈210〉 3
〈211〉 93
〈212〉 DNA
〈213〉 Murinae gen. sp.

〈400〉 3
ctcttagcag gaatttggag atgacttttg atgacaagat gaagcctgcg aatgacgagc 60
ctgatcagaa gtcatgtggc aagaagccta aag 93

〈210〉 4
〈211〉 102
〈212〉 DNA
〈213〉 Murinae gen. sp.

〈400〉 4
gtctgcattt gctttcttcc ccatggtggt tccctgctgc tatgactctg gtcatcctct 60
gcctggtgtt gtcagtgacc cttattgtac agtggacaca at 102

〈210〉 5
〈211〉 246
〈212〉 DNA
〈213〉 Murinae gen. sp.

〈400〉 5

tacgccaggt atctgacctc ttaaaacaat accaagcgaa ccttactcag caggatcgta 60
tcctggaagg gcagatgtta gcccagcaga aggcagaaaa cacttcacag gaatcaaaga 120
aggaactgaa aggaaagata gacaccctca cccagaagct gaacgagaaa tccaaagagc 180
aggaggagct tctacagaag aatcagaacc tccaagaagc cctgcaaaga gctgcaaact 240
cttcag 246

〈210〉 6
〈211〉 138
〈212〉 DNA
〈213〉 Murinae gen. sp.

〈400〉 6

aggagtccca gagagaactc aagggaaaga tagacaccat cacccggaag ctggacgaga 60
aatccaaaga gcaggaggag cttctgcaga tgattcagaa cctccaagaa gccctgcaga 120
gagctgcaaa ctcttcag 138

〈210〉 7
〈211〉 138
〈212〉 DNA
〈213〉 Murinae gen. sp.

〈400〉 7

aggagtccca gagagaactc aagggaaaga tagacaccct caccttgaag ctgaacgaga 60
aatccaaaga gcaggaggag cttctacaga agaatcagaa cctccaagaa gccctgcaaa 120
gagctgcaaa cttttcag 138

〈210〉 8
〈211〉 137
〈212〉 DNA
〈213〉 Murinae gen. sp.

〈400〉 8
gtccttgtcc acaagactgg ctctggcata aagaaaactg ttacctcttc catgggccct 60
ttagctggga aaaaaaccgg cagacctgcc aatctttggg tggccagtta ctacaaatta 120
atggtgcaga tgatctg 137

〈210〉 9
〈211〉 116
〈212〉 DNA
〈213〉 Murinae gen. sp.

〈400〉 9
acattcatct tacaagcaat ttcccatacc acctccccgt tctggattgg attgcatcgg 60
aagaagcctg gccaaccatg gctatgggag aatggaactc ctttgaattt tcaatt 116

〈210〉 10
〈211〉 2582
〈212〉 DNA
〈213〉 Murinae gen. sp.

〈400〉 10
ctttaagacc aggggcgttt ctttacagct atattcatca ggcaactgtg cataccttca 60
agacggagct gtgttcgctg aaaactgcat tctaattgca ttcagcatat gtcagaagaa 120

gacaaatcat ttgcaaattt agtgaatcta aagattctgg agaagaccat gagaagactt 180
ttgactgtcg ctctgaaatt taagctattc tttattacct gcatgtaaag catgttggcc 240
ttgacatctg tcagttacct gatagctaca gttcacctca acaaagacaa ggaccagaag 300
caaatactgg tggatccaga tgtttgaaat ctttgtatca aaacgtgtga gttcaattgt 360
ttatccatat acactggcct tgcccctcca aagtctccca accaacctgc aatccttctt 420
ccccttcgtg ttttaaatga tgcttcctgc ctgacctggc catgctttgt actcagtctc 480
ctctacctca gtatgcctcc tgttgccgca tgaaagacag gatgtagaaa tcctcctcaa 540
gtgcaggcag agggctcaaa ggcaaagctc gtccgagaaa taacatgcaa agaaacagaa 600
ctggaaaaac tacactgcaa acaggaactc atgatctcta aaaagccatg gcttgatctt 660
tatgacagga gtccatctcc agactgcact tttacacaca tatttttat tttcctttta 720
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actgaagatc agaagaggac atcagattct ctggatctgg aattgcagat ggttgtaagc 840
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ccatgagcta tctctcaagc tcctgtgatc aattcttgta tcagttatgt ctccatttt 960
gctctaccaa agaacagtgt tataacttta aaacagtaag tattttcatt attctagggg 1020
tattatagca gatatataga tatagatata gatgatatag atatagatat agatatagat 1080
atagatatag atatagatat agatatagat atagatatag atatagatat agatatagat 1140
atagatatag atatagatat agagtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtt 1200
tctatgctag atacatcctt gagaagatga gacagtttig tgtgaaatga gtttgtaata 1260
atccaaattt aaaaataaat tcgatgatta cctgtagtgg tcatattacc acagctaaga 1320
tgatgaacat acctgtcact tctgcccctt tccaaagcct ccccctaaa acaaacacca 1380
atctgctttc agttcgcatt ttatagagct tatcattttg tttttaagac agaatctcat 1440
tatatagttc tggctggcct ggaactcact acacagagca ggctggcctc caccttctag 1500
agctcctcct gccagactcc caaaccttaa gactgaagga gtgccctgcc atgtgtgact 1560
caaacacctt aatgtgaatg gaatagcata agatgtccag gttttttica gtccagcttc 1620
ttccacttgg tacaattttt aatttttgtg ttcatacatc tccaccacag tgtttgtatc 1680
agttcatcat tcttttcaaa tgttgagcct tcccctgtgg atctatagtg tcatttgtta 1740
tctgtgtatt tgttgatgcg atttgggttg tttttatttg gggtcaccta caaataaagc 1800
tgctatggat gtccatggac aaggctaata tcttaggtaa gcacctacga gtaagatgct 1860

tgggtcattc agtgtgggaa tatatggttg gctattttaa ccattcctgt ttgaaaacat 1920
taatttttt atttttgaaa tcaatttttt aaaaaattag tctattttac atctcaaccc 1980
cagtttctct tcctcctctc ctctcaacct tctcccacct tctctccctg accccatcca 2040
cccctcctcc ctttctctcc agaagagggg aggcctccca tggatgccaa ccagcctcag 2100
catctcaagt tacagtaaga ataggtttgt cttctcttgt gaaaatctta atttttagat 2160
ttatctatta tatatgcagt actttgcttg cacatatgta ttggtaccat gtacatgaat 2220
ggtaccagag aaagtcagaa gaggccattg tattttctgg gactggaatt acagacggtt 2280
ttgaacaatc ctatagactc tgggaactga acccaggtcc tctggaaagg caagcagtgc 2340
tcttaacccc tgagccattt cttctggcct tttagcaatt tttattaata tataactgtg 2400
tataattgca cttttagctc aaagttctta agtgtcaaat agtcttggat ttattttcat 2460
gttatcactg tctgtacaat ttctgtgatg aaataactga gcatatattt tgagaatttg 2520
gttttcttaa attttaaatc tgaaggattt acatatttga gaataaaaac cagccagata 2580
tg 2582

〈210〉 11
〈211〉 22
〈212〉 DNA
〈213〉 Artificial Sequence

〈220〉
〈223〉 Description of Artificial Sequence:sense primer
for mouse Lox-1 cDNA amplification

〈400〉 11
aggtcctigt ccacaagact gg 22

〈210〉 12
〈211〉 23
〈212〉 DNA
〈213〉 Artificial Sequence

〈220〉
〈223〉 Description of Artificial Sequence:antisense primer for mouse LOX-1 cDNA amplification

〈400〉 12
acgccccigg tcttaaagaa ttg 23

〈210〉 13
〈211〉 22
〈212〉 DNA
〈213〉 Artificial Sequence

〈220〉
〈223〉 Description of Artificial Sequence:sense primer for GADPH cDNA amplification

〈400〉 13
gaccacagtc catgacatca ct 22

〈210〉 14
〈211〉 21
〈212〉 DNA

〈213〉 Artificial Sequence

〈220〉

〈223〉 Description of Artificial Sequence:antisense primer for GAPDH cDNA amplification

〈400〉 14

tccaccaccc tgttgctgta g 21

[Brief Description of the Drawings]

[Drawing 1] It is drawing showing from a top the probe for Southern blotting for the restriction enzyme site of mouse LOX-1 gene contained in Clone D, the structure of the vector for homologous recombination, the structure of mouse LOX-1 gene after homologous recombination, and homologous recombination object analysis, respectively.

[Drawing 2] It is drawing showing the result of the Southern hybridization of the embryonic stem cell genome DNA fragment using the probe which has the array of the probe which has the array of the upstream, and 5'3' downstream among the probes of drawing 1 .

[Drawing 3] It is drawing showing the result of the Southern hybridization using the probe which has the array of 5' upstream among the probes of drawing 1 of a mouse genome DNA fragment.

[Drawing 4] Mouse LOX-1 cDNA and mouse GAPDH It is drawing showing the electrophoresis result of the magnification product by RT-PCR of cDNA.

[Drawing 5] It is drawing showing the result of having compared decrease of the vascular endothelial cell dependency relaxation response by oxLDL with the wild type mouse and the LOX-1 genetic-defect mouse.

[Drawing 6] Babesia It is drawing showing the result of having compared PARASHITEMIA after microti infection with the wild type mouse (C57BL/6J) and the LOX-1 genetic-defect mouse (LOX-1 KO).

[Drawing 7] Babesia It is drawing showing the result of having compared PARASHITEMIA after rodhaini infection with the wild type mouse (C57BL/6J) and the LOX-1 genetic-defect mouse (LOX-1 KO).

[Drawing 8] It is drawing showing the result of having measured the guide-peg volume after carrageenin processing with the wild type mouse (WT) and the LOX-1 genetic-defect mouse (KO).

[Drawing 9] The organization photograph of the carrageenin treatment guide-peg root of a wild type mouse (1-3) and a LOX-1 genetic-defect mouse (4-6) is shown. 1 and 4:x50, the photograph (x125) to which 2 and 5 expanded 1 and 4 respectively, and 3 and 6 are the photographs (x250) to which photographs 2 and 5 were expanded respectively. ** shows inflammatory cell infiltration and an arrow head shows an edema.

[Translation done.]

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